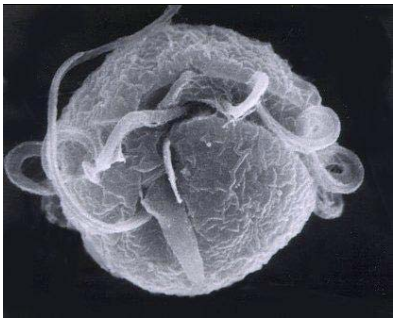
Pfiesteria piscicida



Description: *Pfiesteria piscicida* is a toxic dinoflagellate with multiple life stages. Below is an image of the zoospore stage. *Pfiesteria* and *Pfiesteria*-like organisms are found along the east coast of the United States.

Toxin Produced: Pfiesteria Toxin
The chemical structure for the toxins associated with fish kills and fish lesions has not yet been identified. Research is currently being done to purify the toxin and identify its structure, and to understand the mode of action of the toxin.

Syndrome: Possible Estuary Associated Syndrome (PEAS)
Human health effects of *Pfiesteria piscicida* are neurologic and occur when people come into contact with toxic aerosols and/or toxic water. Symptoms include narcosis, development of sores, headaches, blurred vision, memory loss, confusion, and respiratory problems.

Distribution: **East Coast**: Southern Maine to Northern Florida

Accomplishments: 1998-present

1998: ***Mechanisms of Toxicity***
A water-soluble toxic activity produced by *Pfiesteria piscidia* has been partially purified in collaboration with North Carolina State University. Reporter gene assays and calcium digital image analysis have been used to investigate the mechanism of action of the putative toxin on calcium signaling pathways in pituitary tumor cells. The putative toxin has also been found to have comparable effects on primary cultures of neurons isolated from memory processing region of the brain (hippocampus) of laboratory rats.
Contact: John Ramsdell

2000: Potential Toxicity of *Pfiesteria*-like Dinoflagellates Isolated From Florida
Cultures of different species of "Cryptoperidiniopsoid" dinoflagellates were grown under controlled conditions. Each culture was identified via scanning electron microscopy at the Florida Marine Research Institute before shipment to the Marine Biotoxin Program. Each strain was re-identified after mass culture and toxin analysis. Strains were grown in 100 L batches cultures and harvested at late-log growth phase. Production of biological active substances by each culture was examined from both resulting cell mass and spent culture medium. Both cell mass and spent culture medium were passed though a silica column and eluted with an elutropic solvent series. Totals of 5 samples were collected for both the cell mass extract and spent culture medium. Each of the 10 extracts was tested for the possibility of bioactivity using both live assays and cell based assays. Live bioassays included brine shrimp and sheepshead minnows while cell based assay included the GH4C1 cytotoxicity assay. Solvent fractionation yielded several fractions that were active. A non-polar fraction was active on the shrimp bioassay and the sheepshead minnow bioassay. Subsequent structural analysis of this fraction showed this activity in part was due to DEHP, a man-made phthalate ester. This and other fractions are still under pharmacological characterization. A polar fraction was active on the brine shrimp bioassay and the cytotoxicity assay but was inactive on the sheepshead minnow assay. This data provides initial evidence of bioactive substances from cultures of Cryptoperidiniopsoid. Whether this organism produces a toxic substance is presently

unknown and will require future pharmacological and chemical investigations. *Contact: Steve Morton*

Preparative Isolation of Pfiesteria Toxins From Culture

Methodology for the reproducible isolation of Pfiesteria toxins has been developed and employed for preparative toxin production. This methodology allows toxic fractions to be isolated in a stable form providing a relatively neutral environment free of matrix buffers, enzymes and so forth. Such methodology now enables us to rapidly isolate the toxins from mass culture, quickly removing the toxin(s) from oxidative or reductive environments that would otherwise degrade or destroy the toxin. Purified extracts obtained from this method are being used for toxin characterization both in terms of biological activity as well as molecular characterization using Nuclear Magnetic Resonance and Mass Spectrometry. A lipohpillic toxic fraction has been identified by NMR and GC-MS as bis(diethylhexyl) phthalate, a common plasticizer. The major source for this material has been identified as Instant Ocean. No other non-polar toxin has been observed. The characterization of the polar toxin(s) is well underway.

Contact: Peter Moeller

Receptor Identified For Putative Pfiesteria Toxin Provides Insight Into Effects Of Pfiesteria On Humans And Fish

The pharmacologic activity of a putative toxin (pPfTx) produced by *P. piscicida* has been examined by characterization of the signaling pathways that induce the c-fos luciferase construct in GH_4C_1 rat pituitary cells. A class of purinergic receptors mediates this c-fos pathway with analog selectivity and functional ionic conductances consistent with a purinergic receptor of the P2X7 class. The irreversible P2X7 antagonist, adenosine 5'-triphosphate-2',3'-dialdehyde, was used to demonstrate that the pPfTx requires this pathway for activation. P2X7 receptors are found predominantly on myeloic cells including mature macrophages, mast cells and microglial cells. A role of P2X7 receptors in the action of pPfTx is of interest**,** in consideration of the fact that this toxic dinoflagellate has been reported to cause a range of health impacts in both finfish and humans. The effects linked to *Pfiesteria* toxicity may be related to an inflammatory response, either in macrophages in the periphery or microglia in brain tissue. Implication of P2X7 receptors as a potential target for the bioactive substance produced by toxic *P. piscicida* provides a common basis for the investigation of symptoms that previously have been regarded as unrelated, such as ulcers in menhaden and cognitive dysfunction in humans. *Contact: John Ramsdell*

Publications:

1. Pravasoli-Guillard National Center for Culture of Marine Phytoplankton 1997 list of strains. **J. of Phycology** 33(6): 1-75.
2. Classification, Nomenclature, and Identification of Pfiesteria and Pfiesteria-like Species. **Environmental Health Perspectives**. 109(sup 5):661-665
   Abstract Available
3. Reporter gene assay for fish killing activity produced by *Pfiesteria piscicida*, **Environmental Health Perspectives** 107: 711-714.
4. Reporter gene assays for algal-derived toxins. **Natural Toxins** 6:415-421.
   Abstract Available
5. In vitro detection methods for algal toxins: conceptual approaches and recent developments. JAOAC International 84:1617-25
   Abstract Available
6. Current Progress in isolation and characterization of toxins isolated from Pfiesteria piscicida. **Environmental Health Perspectives**, 109, Suppl. 5 739-744.
   Abstract Available

7. Pfiesteria Toxins GENERAL REFREE REPORTS: Committee on Natural Toxins and Food Allergens Phycotoxins, **JOURNAL OF AOAC INTERNATIONAL** VOL. 84,198-199. Abstract Available
8. Reporter gene assay for fish killing activity produced by *Pfiesteria piscicida*. **Environmental Health Perspectives** 107: 711-714.
9. Identification of a P2X7 receptor in GH4C1 rat pituitary cells: a target for a bioactive substance produced by Pfiesteria piscicida. **Environmental Health Perspectives**, *in press*
10. Pfiesteria Toxins GENERAL REFREE REPORTS: Committee on Natural Toxins and Food Allergens Phycotoxins, **JOURNAL OF AOAC INTERNATIONAL** VOL. 84,198-199. Abstract Available
11. Microflourimetric Analysis of a purinergic receptor (P2X7) in GH4C1 rat pituitary cells: Effects of a bioactive substance produced by Pfiesteria piscicida. **Environmental Health Perspectives**, 109 Suppl. 5, 731-738. Abstract Available
12. Health and Ecological impacts of harmful algal blooms: risk assessment needs. **Human and Ecological Risk Assessment 7**: in press.